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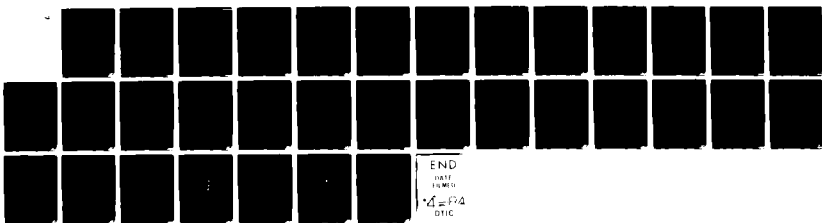
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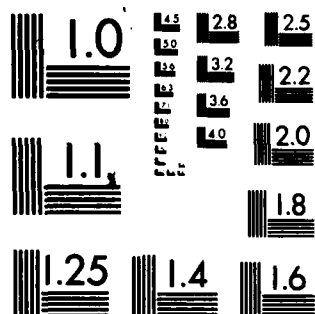
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THE LUNG SURFACTANT SYSTEM IN ADULT RESPIRATORY DISTRESS SYNDROME

Annual Progress Report

John U. Balis

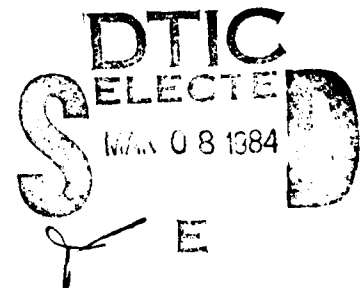
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1. APPLICANT'S ACCOMPLISHMENTS IN RESTRUCTURING THIS RESEARCH PROGRAM

The relocation of the applicant and three key members of his research team to Tampa (July, '78) inevitably resulted in a temporary disruption of our research program. However, this relocation also provided our team with exciting opportunities to enhance our overall future productivity.

Initially, considerable effort was spent in setting up a functional research laboratory for the studies involved in the program. This was accomplished in a relatively short period of time. On the other hand, the lack of a competent clinical team and the need for additional professional and technical personnel restricted considerably our ability to initiate human and animal studies relating to the pathophysiology of surfactant system in ARDS. In addition, our plans to continue experimental work using primates could hardly be implemented due to scarcity of Rhesus monkeys suitable for our studies. We have overcome these difficulties by (1) establishing collaboration with other investigators with appropriate expertise and/or clinical experience, (2) securing the participation of additional technical personnel, and (3) developing a reliable rat model to study structural, functional and biochemical changes of the surfactant system in ARDS.

2. ISOLATION AND CHARACTERIZATION OF SURFACTANT FROM MINCED LUNG TISSUE (1,2-See appended manuscript and abstract)

(A) Rabbit Lung:

→ In our earlier experiments (3) we were unable to demonstrate increased concentration of sIgA in the surfactant fraction of rabbit lung washing, suggesting that the sIgA found in washing represents, at least in part, a contaminant from the tracheo-bronchial passages. 1.3

In order to investigate whether or not sIgA is present in the alveoli, surfactant was obtained by a method designed to substantially reduce contamination with tracheobroncheal constituents. Following removal of visible bronchi, rabbit lung tissue was finely minced in saline, tissue fragments and cells were filtered through nylon bolting cloth and low speed centrifugation, and the supernatant was used to prepare density gradients for the isolation of surfactant as previously described (4). Each fraction of the gradient was examined by SDS-PAGE. The results revealed that sIgA was present only in the surfactant fraction. This investigation suggested that alveoli contain sIgA which is specifically associated with surfactant phospholipid. Further studies indicated that although extracts from minced lung contain greater amounts of contaminating lipids and proteins than lung washing, these contaminants can be readily removed by density gradient centrifugation. No significant differences in phospholipid distribution or phosphatidylcholine (PC) fatty acid composition can be detected in surfactant isolated from minced lung washing and surfactant isolated from alveolar washing. It appears, therefore, that mincing lung tissue is as good or better way to obtain surfactant as lavaging intact lungs. This finding is of particular importance in studying human lung surfactant, since portions of surgically removed lung specimens can be rapidly minced and used as a source of human surfactant.

(B) Human Lung:

The primary objective of my research program relates to the pathophysiology of the surfactant system in ARDS. This important field has been largely neglected due at least in part to the lack of criteria defining the normal human surfactant. At the present time, the ideal source for surfactant is lung washings obtained by alveolar lavage of excised lung specimens. However, it is not possible to obtain fresh (surgically excised) normal human lung suitable for lavaging. Therefore, only tracheal aspirates from patients on respirator as well as post-mortem lung specimens can be used to study the normal surfactant system of adult human lung. We performed previously similar studies of perinatal human surfactant (5). However, the latter studies were considerably facilitated by the fact that amniotic fluid from uncomplicated term pregnancies could be readily used as the source for normal surfactant.

During the course of our studies with rabbit lung, we found that we could purify surfactant from lung tissue minced in saline as readily as from alveolar lavage. Following these results, we have performed a series of studies on grossly and histologically normal human lung tissue from surgically excised lobectomy or pneumonectomy specimens. The procedures are as follows:

The lung tissue is minced with scissors in a beaker of saline. This method of obtaining surface active material is one of the earliest methods described in the literature. The tissue fragments are removed by filtration through Nylon bolting cloth and the cells and debris are pelleted by low speed centrifugation. The supernatant is then used to prepare gradients in the same manner as we used for alveolar lavages. NaBr is added to the minced supernatant to give a concentration of 16%. This is overlaid by a discontinuous density gradient of 13% NaBr in saline and saline with no NaBr. Following centrifugation at 90,000 g for 3 hours, the surfactant is found as a visible, particulate band near the top of the tube. When using mince, repeated centrifugation is needed to remove contaminating protein, so NaBr is added to the surfactant taken from the 1st gradient, to give a concentration of 16% and this is used as the bottom layer in another gradient prepared in the same manner as described above. This mince contains about 15 times as much protein as phospholipid. In the 1st gradient, the surfactant band contains about 35-40% of the phospholipid in the mince, but only 1 to 2 percent of the protein. One gradient centrifugation is probably sufficient for the study of the phospholipids, since on a 2nd gradient, the surfactant band contains 90% or more, the phospholipid present in the gradient. However, the second gradient is quite useful for removal of excess protein since only 10-20% of the protein is recovered in the surfactant fraction. The ratio of phospholipid to protein of the 2nd gradient surfactant is always greater than 10/1. In order to remove the last traces of excess protein, we repeat the gradient centrifugation a 3rd time. This step removes another 25-30% of the protein; and the protein remaining in the surfactant fraction cannot be removed by additional centrifugation.

The surfactant thus purified has fairly consistent phospholipid to protein ratios. In 5 cases, the values ranged from 11.4 to 13.4 with a mean of 12.3 (0.4 SEM). The material is surface active, with a sample containing 25 g of phospholipid reducing the minimum surface tension to less than 12 dynes/cm. The surfactant contains very small amounts of neutral lipids, cholesterol, free fatty acids and triglycerides, and a large amount of phospholipid.

As shown in Table 1, the major phospholipid is phosphatidylcholine (PC) which represents 76% of the total phospholipid. The next most abundant phospholipid is phosphatidylglycerol (PG) which is about 10% of the total. Phosphatidyl inositol (PI), phosphatidyl ethanolamine (PE), phosphatidylserine (PS), sphingomyelin (SPH) and an unidentified phospholipid (MP) are present in small but consistent amounts. The PG content of this surfactant from adult lung is considerably higher than in surfactant from term amniotic fluid (5%) which is in accord with the finding of Hallman et al. (6) that PG does not appear until near term, and in rabbit fetuses, increases rapidly following birth (7).

Shown in Table 2 are the major fatty acids of the surfactant phosphatidylcholine, given as percent of total fatty acids, mean value standard error of the mean. Palmitic acid (16:0) is about 66% of the total. This is somewhat less than we find in perinatal human surfactant which is 74%, but the overall distribution of fatty acids is similar to perinatal surfactant, and is almost identical with that of adult rabbit lung surfactant. This similarity to rabbit surfactant does not extend, however, to the proteins, which were studied by gel electrophoresis. These studies demonstrated that the most abundant proteins of rabbit lung surfactant are secretory IgA, IgG and albumin. In human surfactant, no major band can be seen at the position of sIgA, even though we can detect the presence of IgA by reaction with antiserum to alpha chain on immunodiffusion plates and can detect the secretory component in tissue sections by immunofluorescence. Rather, the major proteins of human surfactant appear to be IgG, albumin and 2 high molecular weight proteins (400,000). We have also examined nonsurfactant fractions of the 2nd gradients used for purification of surfactant and found that they contain IgG and albumin, but not these high molecular weight proteins.

Further data were obtained when human lung surfactant was treated with dithiothreitol (DTT) to reduce disulfide bonds and electrophorized on 5.6% gels with SDS. In these gels, the major band has a MW of about 33,000 and other bands which correspond to the expected relative mobilities of albumin and immunoglobulin heavy and light chains. In addition, another small as yet unidentified band migrates near albumin. In human plasma treated in the same manner, there is virtual absence of any band in the position of the peptide with MW of 33,000. On the other hand, gels of reduced rabbit surfactant reveal much larger bands of immunoglobulin heavy and light chains as compared to the human and much smaller amounts of the 33,000 molecular weight peptide. It is of interest to mention that a peptide with similar molecular weight has been reported for dog lung surfactant (8). Recent studies using two-dimensional electrophoresis on slab gels have revealed that both high molecular weight bands in human lung surfactant upon reduction, yield two peptides, one of about 35,000 daltons and the other about 70,000.

In summary, our results with human lung surfactant support the concept that surfactant contains specifically associated protein. We found two proteins with molecular weights of more than 400,000 and following reduction with DTT, peptide subunits with molecular weights of 33,000. A distinct species difference is seen in rabbit surfactant, which contains a small amount of the 33,000 molecular weight peptide but a large amount of sIgA. By contrast, rabbit surfactant phospholipids show a remarkable similarity to phospholipids of human surfactant. Finally, this study demonstrates that our method of isolating surfactant from minced lung tissue is quite adequate for normal human lung and our findings provide a basis for further evaluation of surfactant alterations in ARDS and other pathologic conditions of the human lung.

3. THE SURFACTANT SYSTEM IN ARDS

This study involves correlation of changes in the composition of purified surfactant from tracheal aspirates and post mortem lung specimens with clinical and morphologic parameters relating to the severity, progression and possibly treatment of ARDS.

A prerequisite for the success of such studies is the establishment of clinical criteria for the selection and follow-up of patients with or without ARDS. This has been accomplished with the assistance of a competent clinical team of physicians who are now actively participating in the program. The clinical studies are directed by Dr. Allen Goldman, Chief, Pulmonary Disease Section and Associate Professor, Department of Medicine, University of South Florida. We have already isolated surfactant present in tracheal aspirates from 6 patients without ARDS. Preliminary studies indicate that the phospholipid composition of surfactant from tracheal aspirates is similar to that obtained from human minced lung tissue.

4. MECHANISMS OF CONGESTIVE ATELECTASIS IN ENDOTOXIN SHOCK (9)

Rhesus monkeys infused with E. Coli endotoxin (10 mg/kg/hr) for up to 22 hours develop progressive pulmonary lesions including pulmonary leukocytosis with sustained phagocytosis of endotoxin by the marginating leukocytes, endothelial cell damage, edema and congestive atelectasis. Further ultrastructural studies demonstrated multifocal swelling of type I epithelium, marked foldings of alveolar walls at sites of epithelial damage and focal deposition of surfactant material sealing the openings of plicated type I cells. These changes were best appreciated in lung specimens fixed during the expiratory phase with procedures designed to preserve alveolar architecture and the surfactant layer. Epithelial injury appeared especially prominent following prolonged assisted ventilation with room air. Lung washings from endotoxin infused monkeys contained an excessive amount of protein, reflecting increased permeability of the blood-air barrier. Purified surfactant fractions of the washings were not appreciably altered with respect to surface activity, phospholipid/protein ratio, phospholipid distribution and fatty acid composition of phosphatidylcholine. The results indicate that multifocal damage of the injury-sensitive type I epithelium promotes extensive expiratory collapse, due to the inability of surfactant layer to form at sites of epithelial damage.

5. LIGATION OF RAT CECUM AS A MODEL FOR DAD

Our initial plans to further evaluate our primate model for shock lung were postponed indefinitely because it has become virtually impossible to procure suitable Rhesus monkeys for these experiments. For this reason, we have recently performed a series of pilot experiments which have enabled us to develop a reliable rat model for DAD.

In this model, the standard procedure involves ligation of the distal 2 cm of the rat cecum under aseptic conditions. This procedure results in gangrene of the ligated cecal segment with abscess formation and peritonitis. Survival in the above model is largely dependent upon the size of the ligated cecal segment. Following total cecal ligation, the rats uniformly die within 24 hours. With the standard procedure, the survival rate is about 60% and 40% at 24 and 120 hours respectively. In rats killed at 24, 48, 72 and 120 hours, gram negative bacteria (E. coli, Bacteroides fragilis or Klebsiella) were consistently isolated from the peritoneum, blood, lung, liver and kidney. Histologic and electron microscopic

evaluation of lung tissue revealed progressive congestive atelectasis, margination of leukocytes and prominent endothelial damage in association with edema which was primarily interstitial. Moreover, sequential alveolar reactions were consistently observed, and these included damage of the type I epithelium, increase accumulation of alveolar macrophages and, at 72-120 hours, striking increase in the number of type II cells as well as infiltration of the septa with lymphoid and histiocytic cells. The above histopathologic changes are similar to those seen in DAD or interstitial pneumonitis. Therefore, ligation of rat cecum appears to be an excellent animal model for gram negative sepsis and ARDS.

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Table 1. Purified human surfactant

| PERCENT OF TOTAL PHOSPHOLIPIDS | | | |
|--------------------------------|------------|-----|-----------|
| PC | 76.4 ± 1.0 | PS | 1.8 ± 0.1 |
| PG | 10.2 ± 0.9 | SPH | 1.2 ± 0.2 |
| PI | 4.0 ± 0.7 | | |
| PE | 4.1 ± 0.3 | NI | 2.3 ± 0.5 |

Table 2. Purified human surfactant

| MAJOR FATTY ACIDS OF PC | | | |
|-------------------------|------------|-------------|------------|
| <u>14:0</u> | 1.8 ± 0.6 | <u>18:0</u> | 4.0 ± 0.6 |
| <u>16:0</u> | 65.7 ± 2.7 | <u>18:1</u> | 14.2 ± 2.3 |
| <u>16:1</u> | 8.3 ± 0.3 | <u>18:2</u> | 4.9 ± 0.6 |
| | | <u>20:4</u> | 1.1 ± 0.2 |

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SECRETORY IgA - A MAJOR PROTEIN COMPONENT
OF RABBIT LUNG SURFACTANT

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SUMMARY

Secretory IgA is a major protein component of rabbit lung surfactant purified by NaBr density gradient centrifugation from endobronchial lavage and minced lung tissue. Secretory IgA was found in both surfactant and non-surfactant fractions obtained from endobronchial lung washings. By contrast in minced-lung washings, which are not contaminated with proteins from the upper respiratory tree, sIgA is prominent only in the surfactant fraction. These findings indicate that in rabbit lung sIgA is present in the alveoli and is intimately associated with the surfactant system.

INTRODUCTION

Lung surfactant is a phospholipid-rich, surface active substance which lines the alveoli of mammalian lungs and functions as an alveolar stabilizer by lowering the surface tension at the air-liquid interface. The association of proteins with lung surfactant is as yet a controversial issue [1-9].

Rabbit lung washings and surfactant fractions contain several plasma proteins, with albumin and IgG accounting for the largest percent of the total protein [6,10]. A major non-plasma protein has been identified as secretory IgA (sIgA) [7,10,11]. Whether these proteins are integral components of the surfactant system or represent contamination from the vascular system or tracheobronchial tree during endobronchial lavage has not been established. Based on immunofluorescent studies, Colacicco et al. [7] reported that sIgA was not present in the alveolar lining layer. We previously observed that sIgA was found in all fractions of NaBr density gradients prepared from rabbit endobronchial lung washings [11], which was consistent with the suggestion that sIgA is, at least in part, a contaminant from the tracheobronchial tree. Therefore, in order to investigate whether or not sIgA is present in the alveoli, it is desirable to eliminate contamination of the lung washings with constituents of the upper respiratory

tract prior to analysis of the surfactant proteins. In this study we obtain alveolar washings by mincing lung tissue devoid of major bronchi, and demonstrate that sIgA is an alveolar protein and concentrated in the surfactant fraction.

MATERIALS AND METHODS

Tissue and washings were obtained from freshly excised lungs of male New Zealand White rabbits weighing from 3 to 5 kg. Following intravenous injection with a lethal dose of sodium pentobarbital, the abdominal aorta was transected, and the lungs, heart and trachea removed en bloc.

Alveolar washings were obtained from lung tissue devoid of visible bronchi by the following procedure performed at room temperature. The lung was minced, using scissors, in 60 ml of isotonic saline until the fragments were approximately 3 to 5 mm cubes. This suspension was then filtered through nylon bolting cloth (HC160, Tetco) which retained the tissue fragments. These fragments were reagentated with another 60 ml of isotonic saline, filtered and combined with the first extract. In selected experiments we followed the same procedure but used tissue from only subpleural portions of the lung in order to virtually eliminate any possible contamination of the alveolar washing with bronchial

constituents. Endobronchial lung washings were obtained by two consecutive lavages with 70 ml of isotonic saline at room temperature. Cells and debris were removed from minced-lung washings and endobronchial lung washings by centrifugation at $480 \times g$ for 10 min and the supernate stored at -70°C .

Surfactant was isolated from the washings using NaBr density gradient centrifugation in a Spinco SW 25.2 rotor as previously described [12]. After centrifugation, 3 ml fractions were collected by puncturing the bottom of the gradient tube with a needle held in a plastic cylinder. For further purification, the NaBr concentration of the fractions containing surfactant was adjusted to 16% and this solution was used as the bottom layer in a second density gradient.

Lipids were extracted using $\text{CHCl}_3:\text{CH}_3\text{OH}$ (2:1) [13]. The total lipid phosphorus content was measured [14] and the phospholipid content calculated assuming that 4% of the phospholipid is phosphorus. The phospholipids were separated by two-dimensional thin layer chromatography on silica gel G plates developed in $\text{CHCl}_3:\text{CH}_3\text{OH}:\text{NH}_4\text{OH}$ (140:50:7) in the first dimension and $\text{CHCl}_3:\text{CH}_3\text{OH}:(\text{CH}_3)_2\text{CO}:\text{CH}_3\text{COOH}:\text{H}_2\text{O}$ (50:10:20:10:5) in the second dimension. For phos-

pholipid distribution, the plate was sprayed with 50% H_2SO_4 and charred on a hot plate and each spot scraped into a tube. After digestion of the lipids with HClO_4 , the method of Vaskovsky was followed using working reagent I [15]. The fatty acid composition of the phosphatidylcholine was determined by gas liquid chromatography as previously described [12].

Protein concentrations were estimated by the method of Lowry et al. [16] using human serum albumin as the standard. Gradient fractions were pooled into groups of three after phospholipid and protein determinations, and dialyzed against saline to remove NaBr before delipidation. Due to interference of lipids in gel electrophoresis especially when the phospholipid to protein ratio is high, all samples were delipidated using diisopropyl ether: butanol (6:4) [17]. The aqueous phase was dialyzed against H_2O and the samples were lyophilized and stored at 4°C until further use.

Polyacrylamide gel electrophoresis (PAGE) was carried out according to the method of Fairbanks et al. [18]. Gels of 4% and 5.6% polyacrylamide in the presence of 1% sodium dodecyl sulfate (SDS) were used with and without dithiothreitol respectively. IgG and bovine serum albumin from Sigma were used as standards.

Proteins were further identified by their antigenicity using immunoelectrophoresis (IEP) in the second dimension following SDS-PAGE in the first dimension. This approach was based on the method of Kirkpatrick and Rose [19]; two 4% gels each containing 100 µg of protein were run simultaneously, one gel was stained with Coomassie blue and scanned while the other was sliced laterally into 1.5 mm slices. The slices were placed in serial order on 8 x 10 cm glass slides, then covered with 0.75% agarose (BioRad) containing 1% Triton X-100 (Sigma) in gel buffer, pH 8.4 without SDS. An area of the agarose containing Triton X-100, 1.8 cm wide and located 0.5 cm above the gel slices was removed and replaced with 4.0 ml of 0.75% agarose containing 0.4 ml of antibody (1.7 - 2.0 mg Ab/ml). The plates were electrophoresed at 20 mA/plate for 16 hrs, soaked in 0.9% NaCl for 24 hrs, distilled water for 5 hrs, dried and stained with Coomassie blue [20]. Antibodies to rabbit IgG (heavy chain specific) and albumin were obtained from Cappel and anti-rabbit IgA (heavy chain specific) from Miles Lab.

RESULTS

Density gradient centrifugation of minced lung washings yielded a visible band of particulate material located at the same density as surfactant

from endobronchial lung washings [12]. The amount of phospholipid and protein distributed throughout a representative first gradient of minced-lung washings is shown in Fig. 1. The fractions corresponding to the visible band contained the highest concentration of phospholipid, greater than 55% of the total phospholipid and only about 1% of the protein resulting in a phospholipid to protein ratio of 11/1 (± 1 S.E.). The surfactant fraction was further purified on a second density gradient in order to remove excess protein originating from the highly contaminated minced-lung washing. This recentrifugation resulted in an increase of the surfactant phospholipid to protein ratio to 19/1 (± 1 S.E.), due to the removal of approximately 50% of the protein and less than 5% of phospholipid from the first gradient surfactant fraction (Fig. 2). The distribution of phospholipids (Table 1) and the fatty acid composition of phosphatidylcholine (Table 2) in surfactant purified from minced lung were the same as for surfactant from endobronchial lung washings.

Proteins from all fractions of the first gradient of the minced-lung washings were evaluated by SDS-PAGE on 4% gels. The spectrophotometric scans of these gels are presented in Fig. 1 corresponding to the respective positions of the fractions in the

gradient tube. Bands with the expected mobility of albumin and IgG were the most prominent proteins and present in all of the fractions. A distinct band of a high molecular weight protein with the relative mobility of sIgA [21] was found only in the surfactant fraction.

The surfactant obtained from peripheral lung tissue demonstrated the same protein pattern as the surfactant from whole, minced lung tissue. It is pertinent to interject that sIgA is not prominent in surfactant obtained from human lung tissue [22 and unpublished observations] although intrapulmonary bronchi are more prominent in human lung than in rabbit lung. Following the second gradient centrifugation of minced-lung surfactant the amount of sIgA in the surfactant increased relative to the amount of IgG and albumin (Fig. 3). The same increase in the ratio of sIgA to the other two proteins was also seen in endobronchial lung surfactant from a second gradient.

The identity of the proteins in the surfactant fractions was confirmed using SDS-PAGE/IEP in concert. When minced-lung surfactant was run against anti-rabbit IgA the slices corresponding to the high molecular weight protein's R_f value gave a strong positive reaction (Fig. 4). Similarly, when minced-lung surfactant was run against anti-rabbit IgG and

anti-rabbit albumin, positive reactions were seen in the sections corresponding to IgG and albumin's respective R_f values.

The peptides resulting from treatment of minced-lung surfactant with dithiothreitol, migrated into 5.6% gels as seen in Fig. 5. The major bands correspond to immunoglobulin heavy and light chains, albumin, one peptide with the mobility expected for secretory component and another with a molecular weight of about 34,000.

DISCUSSION

The results of this investigation clearly indicate that the distal air passages of rabbit lung contain sIgA which is intimately associated with the surfactant system. This conclusion was reached by demonstrating a selective concentration of sIgA in surfactant isolated from minced lung tissue. The observed association of sIgA with surfactant phospholipids was apparently masked in our previous studies performed on endobronchial lung washings [11] which, by nature of the lavage procedure, were contaminated with bronchial secretions. This interpretation is supported by finding that repeated gradient centrifugation of the surfactant fraction from either minced-lung washings or endobronchial lung washings resulted in the progressive enrichment of the surfactant phospholipids with sIgA.

A peptide with a molecular weight of approximately 34,000, probably the same as that found in various mammalian species [8,9,23-26], was present in rabbit minced lung surfactant following reduction of the proteins with dithiothreitol. Using procedures described in this report we have recently observed distinct species differences in the protein composition between rabbit and human lung surfactant [22 and unpublished observations]. The protein composition of human lung surfactant included only a small amount of sIgA and larger amounts of two proteins with molecular weights greater than 400,000, as well as IgG and albumin. Following reduction of the human lung surfactant proteins with dithiothreitol the 34,000 dalton peptide was the most prominent protein subunit.

The relationships of these proteins to the surface active phospholipids are not clearly understood. There is evidence that various proteins including serum proteins are normally present in the hypophase of the alveolar lining layer [27]. These proteins may be important in the transport of intracellular surfactant to the surface active monolayer as well as in providing a subphase which promotes formation of a stable surface film. Although a final decision cannot be made as to the role of sIgA and other proteins in the surfactant system, it is

possible that in rabbit lung the sIgA, in addition to immunological activity, may replace at least in part the functional role of other non-plasma proteins found in various mammalian species.

Finally the results of this study demonstrate that the method of obtaining surfactant by mincing lung tissue in saline is as good as or better than obtaining surfactant by lavaging intact lungs. This finding is of particular importance in studying human lung surfactant, since portions of surgically removed lung specimens can be readily obtained, rapidly minced and used as a source of human surfactant.

FIGURE LEGENDS

Fig. 1.

Distribution of phospholipid o o and protein o---o following NaBr density gradient centrifugation of minced-lung washings. Upper section shows SDS-PAGE scans of proteins from corresponding gradient fractions on 4% gels each with 60 μ g protein. Albumin and IgG found in all fractions, sIgA only in surfactant fraction.

Fig. 2.

Distribution of phospholipid o o and protein o---o following a second NaBr density gradient centrifugation of minced-lung surfactant.

Fig. 3.

SDS-PAGE of delipidated minced-lung surfactant from a second gradient on a 4% gel without reduction of disulfide bonds.

Fig. 4.

Immunoelectrophoresis of minced-lung surfactant proteins against anti-rabbit IgA (α chain specific) following SDS-PAGE. Positive reaction corresponds to the protein with a molecular weight of 350,000.

Fig. 5.

SDS-PAGE of delipidated minced-lung surfactant
from a second gradient on a 5.6% gel following
reduction with dithiothreitol.

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TABLE I

Surfactant Phospholipid Distribution

| | Percent of Total Phospholipids ^a | | | | |
|-----------------------------|---|--------------|--------------|--------------|------------------------------|
| | PC | PG | PE | PS | PI UNIDENTIFIED |
| Minced-lung washings | 85.4 (0.8) ^b | 8.2 (0.7) | 2.8 (0.2) | 0.3 (0.1) | 2.3 (0.2) 0.9 (0.2) |
| Endobronchial lung washings | 84.7 (1.3) | 8.8 (0.7) | 3.0 (0.6) | 0.1 (0.1) | 2.4 (0.1) 0.9 (0.3) |

^aPC = phosphatidyl choline, PG = phosphatidyl glycerol, PE = phosphatidyl ethanolamine,
PS = phosphatidyl serine, PI = phosphatidyl inositol

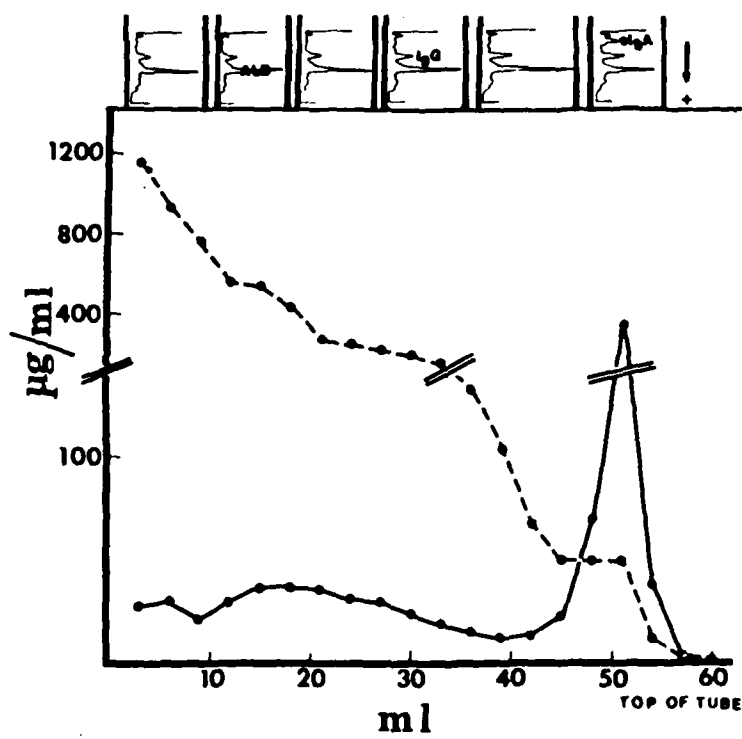
^bNumbers in parentheses indicate \pm S.E.

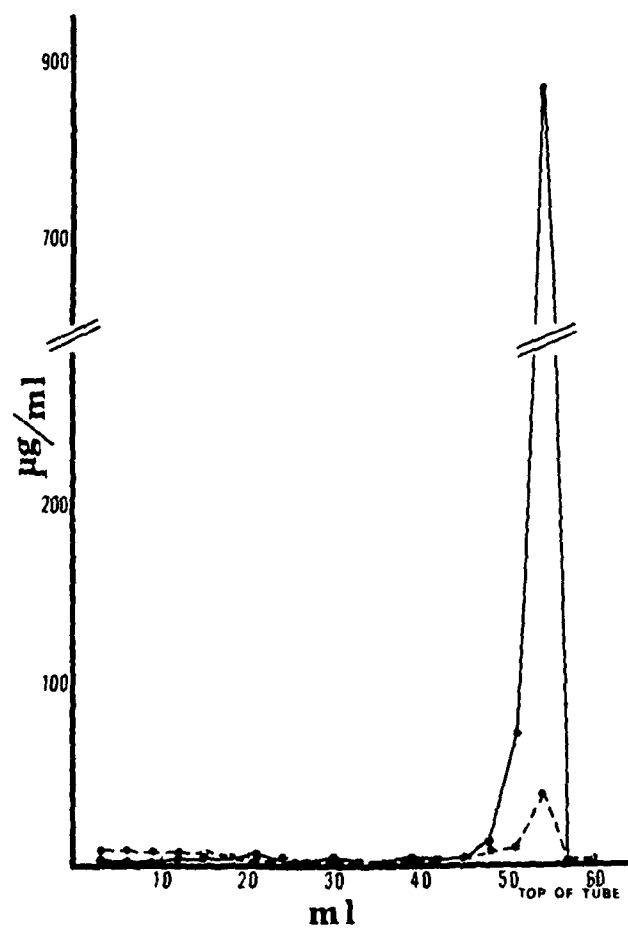
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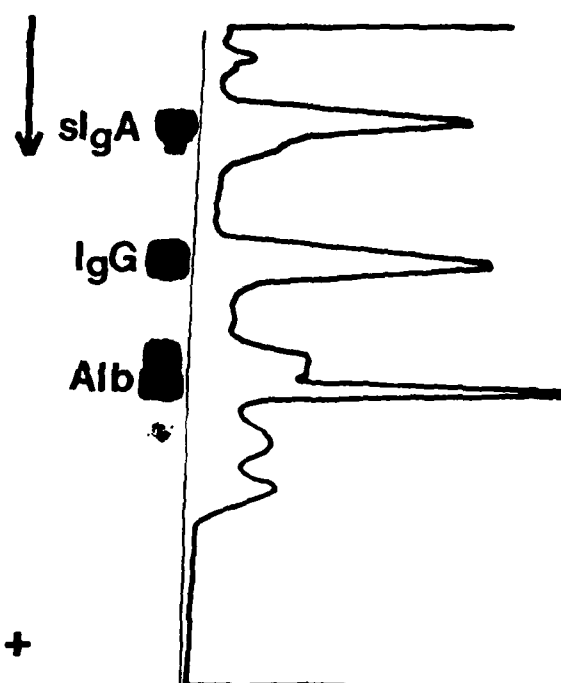
Major Fatty Acids of Phosphatidylcholine

| | Percent of Total Fatty Acids | | | | | | |
|---|------------------------------|---------------|--------------|--------------|---------------|--------------|--------------|
| | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 |
| Surfactant from minced-lung washings | 2.4 (0.3) ^a | 68.7 (0.5) | 5.8 (0.3) | 1.4 (0.2) | 13.7 (0.7) | 7.3 (0.6) | 0.7 (0.1) |
| Surfactant from endobronchial lung washings | 2.7 (0.5) | 67.8 (1.7) | 5.6 (0.3) | 1.7 (0.2) | 14.4 (1.1) | 6.9 (0.9) | 0.9 (0.1) |

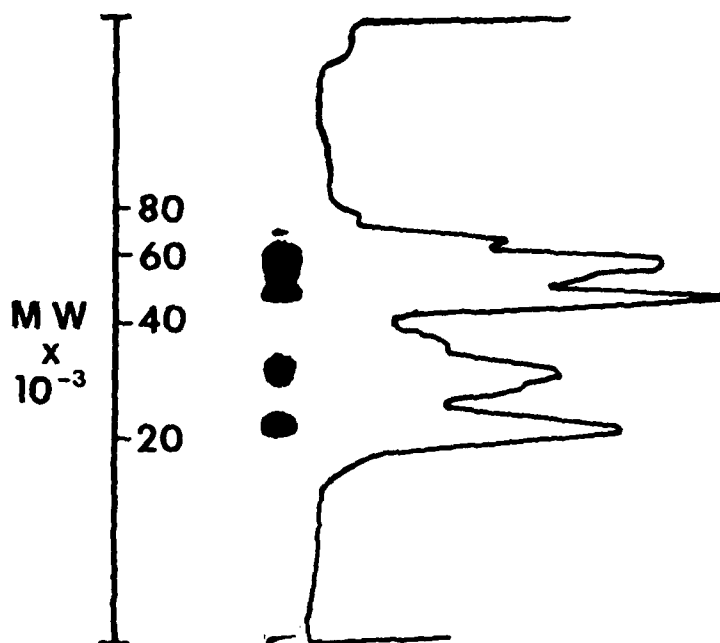
a) Numbers in parentheses indicate \pm S.E.







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CHARACTERIZATION OF THE HUMAN SURFACTANT SYSTEM. Sue A. Shelley\*, June E. Paciga\*, Alan V. Richman\* and John U. Balis. University of South Florida and Tampa V.A.H., Tampa, FL 33612

Since little information is available concerning human surfactant, lung tissue from surgically excised human lungs was minced in saline and surfactant isolated by repeated centrifugation on NaBr density gradients. The surfactant contained 10 times more phospholipid than protein. The most abundant phospholipids were phosphatidylcholine (PC) and phosphatidylglycerol which comprised 76% and 12% of the total phospholipids, respectively. The PC fatty acids included 67% palmitic acid and had a distribution very similar to that of surfactant from human newborn lung washings and term amniotic fluids. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of delipidated surfactant revealed bands with the mobilities of albumin, IgG and secretory IgA (sIgA), as well as a large band of high molecular weight (>400,000) material, which following reduction with dithiothreitol yielded a 37,000 dalton peptide. Immunological studies confirmed the presence of albumin, IgG and sIgA in surfactant. Immunofluorescence studies demonstrated the localization of sIgA secretory component in the alveolar epithelium. The results suggest that sIgA and other proteins represent a small but a consistent and potentially important component of human surfactant. The ability to isolate surfactant from minced lung tissue extends the opportunity to study human surfactant from both normal and diseased lungs. (Supported in part by DAMD 17-78-C-8078, NIH-HD 04434 and MRS-VA)

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